AMENDMENTS TO THE DRAWINGS:

Please substitute the attached replacement formal drawings, Figures 30 and 31, for the original informal drawings in the application. The replacement drawings do not introduce new matter.

REMARKS

Claims 1-54 are pending in the application. Claims 18-21 are withdrawn from consideration as being drawn to non-elected matter. Applicants acknowledge with appreciation the Examiner's rejoinder of Groups 1 and 4-7. Claims 2-17, 22-29, and 31-54 have been cancelled. Claims 1 and 30 have been amended to more particularly point out Applicants' invention and to facilitate prosecution. New claims 55-76 have been added. Support for the new claims can be found on pages 11, lines 37 to 39, to page 12 lines 1-2; page 15, lines 25-38; page 16, lines 1-39 to page 17, lines 1-14; page 20, lines 38-39 to page 21, lines 1-9; page 26, lines 14-27; page 36, lines 14-30; page 37, lines 21-24; page 56, lines 9-33; page 57, lines 10-30; page 37, lines 26-36; page 40, lines 34-39; page 41, lines 1-11; and throughout the application and claims as filed. The corresponding paragraph numbers in the published application are: [0027], [0041]-[0047], [0065]-[0067], [0085]-[0086], [0125]-[0127], [0129], [130], [0143], [0206], and [0208]-[0210].

The specification has been amended to delete reference to embedded hyperlinks, to correct grammatical errors, to insert the complete name and address of the depository of hybridoma, and to add appropriate section headings to the specification. No new matter is believed to have been introduced.

Objections

Claim 1 has been amended to correct the typographical error "human insulin-like growth factor T receptor" with "human insulin-like growth factor I receptor" as suggested by the Examiner.

Claims reciting "one of its functional fragments" have been amended to recite "a binding fragment thereof" as suggested by the Examiner.

Claims reciting "anti-EGFR antibodies, or their functional fragments" have been amended to recite "an anti-EGFR antibody or binding fragment thereof" as suggested by the Examiner.

Based on the above amendments, the objections may be properly withdrawn.

Rejection under 35 U.S.C. §112

Claims 1-17 and 22-54 have been rejected under 35 U.S.C. §112, first paragraph, as purportedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Specifically, the Examiner is requiring deposit of the hybridoma at the CNCM under the number I-2717 as recited in claims 9 and 10. Applicants have cancelled claims 9 and 10, thus rendering the rejection moot.

With respect to corresponding new claim 56, Applicants submit herewith a declaration, in accordance with 37 CFR §1.808, stating that the hybridoma secreting said antibody have been deposited under the Budapest Treaty and that the hybridoma will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. Applicants have amended the specification to recite the date of deposit for hybridoma at the CNCM under I-2717. Furthermore, Applicants submit that the deposit is not necessary to satisfy the enablement and written description requirements under 35 U.S.C. §112, first paragraph for antibodies claimed in, for example, claim 1.

The Examiner also purports that it would require undue experimentation of one skilled in the art to practice the claimed invention. With the amendments made to claims 1 and 30, and cancellation of claims 2-17 31-54, Applicants believe that the rejections are obviated and thus the rejections may be properly withdrawn.

Claims 1-17 and 22-54 are rejected under 35 U.S.C. §112, first paragraph, as purportedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. With the amendments made to claims 1 and 30, and cancellation of claims 2-17 and 31-54, Applicants believe that the rejections are obviated and thus the rejections may be properly withdrawn.

Claims 1-8, 11-17 and 22-54 are rejected under 35 U.S.C. §112, second paragraph, as purportedly being indefinite for failing to particularly point out and distinctly claim the subject matter which application regards as the invention.

Claim 1 is rejected as purportedly ambiguous and indefinite due to the term "if necessary." Applicants have deleted reference to "if necessary", according to the Examiner's suggestion, thus rendering the rejection moot.

Claim 6 is rejected as purportedly ambiguous and indefinite due to the term "significant manner". Applicants have cancelled claim 6, thus rendering the rejection moot.

Claims 7 and 34 are rejected due to the recitation of "...or any fragment whose half-life would have been increased such as pegylated fragments" as

purportedly having no antecedent basis in base claims 1 and 32, respectively. Applicants have cancelled claims 7 and 34, thus rendering the rejection moot.

Claims 2, 3, 4, 5, 11, and 16 are rejected as purportedly ambiguous and indefinite. Applicants have cancelled claims 2, 3, 4, 5, 11, and 16, thus rendering the rejection moot.

Claim 8 is rejected due to the recitation "...murine hybridoma capable of secreting an antibody", as one of ordinary skill in the art cannot appraise the metes and bounds of the claimed invention. Applicants have cancelled claim 8, thus rendering the rejection moot.

Claim 12 is rejected as purportedly ambiguous and indefinite due to the term "moreover comprises". Applicants have cancelled claim 12, thus rendering the rejection moot.

Claim 22 is rejected as purportedly ambiguous and indefinite as to the recitation "...culture of a cell". Applicants have cancelled claim 22, thus rendering the rejection moot.

Claim 24 is rejected as purportedly ambiguous and indefinite as to the recitation "...**moreover**, capable of attaching specifically to the human epidermal growth factor receptor...EGFR". Applicants have cancelled claim 24, thus rendering the rejection moot.

Claim 33 is rejected as purportedly not correlating with the plural antibodies in claim 33. Applicants have cancelled claim 33, thus rendering the rejection moot.

Claim 33 is rejected as purportedly having no antecedent basis in base claim 32. Applicants have cancelled claims 32 and 33, thus rendering the rejection moot.

Claims 25 and 27 are rejected as purportedly ambiguous and indefinite. Applicants have cancelled claims 25 and 27, thus rendering the rejection moot.

Claim 30 is rejected due to the term "composition" as written. Applicants have amended claim 30, according to the suggestion by the Examiner.

Claim 34 is rejected as purportedly ambiguous and indefinite due to the recitation "like pegylated fragments". Applicants have cancelled claim 34, thus rendering the rejection moot.

Claim 37 is rejected as purportedly indefinite and ambiguous due to the recitation "or else". Applicants have cancelled claim 37, thus rendering the rejection moot.

Claim 38 is rejected as purportedly indefinite and ambiguous, due to the recitations "derived natural agents" and "or else". Applicants have cancelled claim 38, thus rendering the rejection moot.

Claim 41 is rejected as purportedly indefinite and ambiguous due to the recitation "antibody compound". Applicants have cancelled claim 41, thus rendering the rejection moot.

Claim 43 is rejected as purportedly lacking antecedent basis in base claim 30. Applicants have cancelled claim 43, thus rendering the rejection moot.

Claim 46 is rejected as purportedly lacking antecedent basis in base claim 45. Applicants have cancelled claim 46, thus rendering the rejection moot.

Claims 47 and 48 are rejected as purportedly indefinite and ambiguous. Applicants have cancelled claims 47 and 48, thus rendering the rejection moot.

Claim 52 is rejected as purportedly indefinite and ambiguous. Applicants have cancelled claim 52, thus rendering the rejection moot.

Claim 53 is rejected as purportedly indefinite and ambiguous. Applicants have cancelled claim 53, thus rendering the rejection moot.

Claims 45-51 and 54 are rejected under 35 U.S.C. §112, second paragraph, as purportedly being incomplete for omitting essential steps, such omission amounting to a gap between the steps. Applicants have cancelled claims 45-51 and 54, thus rendering the rejection moot.

Rejection under 35 U.S.C. § 102

Claims 1, 4, 6, 15, 22-24, 30, 44-52 and 54 have been rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by U.S. Patent No. 5,891,996, issued April, 1999, as evidenced by Rodeck et al (J. Cell Biochem 35(4):315-20, December, 1987. Applicants respectfully traverse this rejection.

The Examiner purports that the '996 patent teaches various antibodies such as humanized, chimeric and monoclonal antibodies against human EGF receptor and a method of making the referenced antibodies, as evidenced by the teachings of Rodeck et al. Further, the Examiner purports that Rodeck et al teach human EGF receptor is a human type I insulin-like growth factor (IGF) receptor, and the referenced antibodies inherently also bind to the claimed human insulin-like growth factor I receptor and inhibit the natural ligand such as IGF1 and IGF2 from binding to its IGF-IR.

Applicants respectfully bring to the attention of the Examiner that EGF receptor is not a human IGF-IR, and anti-EGF receptor antibodies do not inherently also bind to IGF-IR. Applicants also point out that Rodeck et al only teaches that 3

different monoclonal antibodies (anti-EGF receptor, anti-IGF-IR, and anti-NGF receptor) are used in the interactions study between these growth factor receptors. In support of Applicant's position, Garrett et al, *Nature*, 1998, 394 (6691), 395-9, teaches EGFR is only closely related to the insulin receptor (IR) family, which is itself closely related to IGF-IR family, due to the fact that they all belong to the "tyrosine-kinase receptor" genus. Garrett et al. is attached hereto as Exhibit "A".

Furthermore, to demonstrate that EGFR antibodies do not inherently bind to the IGF-IR, Wu X, et al, *J. Clin. Invest.* 1995, 95(40), 1897-1905, indicates that anti-IGF-IR monoclonal antibodies can block the IGF-IR and thus the capacity of insulin/IGF-IR to delay apoptosis induces by anti-EGFR antibodies (mAb 225) when anti-EGFR mAb cannot. Therefore, in view of Wu X et al, antibodies against EGF receptor do not inherently bind to IGF-IR receptor and do not inhibit IGF1 from binding to its IGF-IR (see the paragraph "Addition of IGF-1 or high concentrations of insulin can delay apoptosis induced by mAb 225"). Wu X et al. is attached hereto as Exhibit "B".

Claims 1, 6-8, 11 and 22-23 have been rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by U.S. Patent No. 5,935,821, issued August,1999.

The Examiner purports that the '821 patent teaches monoclonal antibody comprising a light chain having a sequence such as SEQ ID NO:66 that is 93.4% identical to the claimed SEQ ID NO:54, which is at least 80% identical to the claimed SEQ ID NO:54. The Examiner further purports that the referenced antibody inherently also binds (cross-reactive) to the claimed human insulin-like growth factor T receptor IGF-IR. According to the Examiner, the '821 patent also teaches a

method of making the referenced antibody and antibody fragments, and the antibody or its binding fragments do not bind in a significant manner to the human insulin receptor IR since it binds specifically to the paratope of antibody that binds to ganglioside GD2.

Claims 1, 15 and 16 have been rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by U.S. Patent No. 5,608,039, issued March 4, 1997.

The Examiner purports that the "039 patent teaches an antibody such as humanized antibody and binding fragment thereof comprising a light chain sequence such as SEQ ID NO:50 that has a 94.1% sequence identity with the claimed sequences of SEQ ID NO:61 and SEQ ID NO:65, which is at least 80% identical to the claimed SEQ ID NO:61 and 65, for treating cancer. The Examiner further purports that the referenced antibody inherently also binds (cross-reactive) to the claimed human insulin-like growth factor T receptor IGF-IR. According to the Examiner, the referenced humanized antibody inherently comprises FR1 to FR4 of human antibody and light and heavy chain and, thus, anticipates the claimed invention.

Claims 1, 8 and 11-16 have been rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by U.S. Patent No. 6,068,841, issued May 2000.

The Examiner purports that the '841 patent teaches a monoclonal antibody comprising a heavy chain sequence such as SEQ ID NO:11 that has a 86.5% sequence identity with the claimed sequence of SEQ ID NO:69, which is at least 80% identical to the claimed SEQ ID NO:69. The Examiner further purports that the referenced antibody inherently also binds (cross-reactive) to the claimed human insulin-like growth factor T receptor IGF-IR, given the level of sequence identity. The

Examiner further purports that the reference also teaches chimeric antibody and humanized antibody comprising the variable region of the mouse monoclonal antibody, and the light chain and heavy chain constant region derived from human, which is heterologous to the mouse. Additionally, the referenced antibody has a kappa light chain and a heavy chain of IgG2. According to the Examiner, the '841 patent teaches various hybridoma that are capable of secreting the referenced antibodies, and thus the referenced teachings anticipate the claimed invention.

Claims 1, and 15-16 have been rejected under 35 U.S.C. § 102(e) as being allegedly anticipated by U.S. Patent No. 6,300,064 B1, filed August 19, 1996.

The Examiner alleges the '064 patent teaches an antibody that comprised a heavy chain sequence such as SEQ ID NO:39 that is 81.9% identical to the claimed SEQ ID NO:83. The Examiner further purports that the referenced antibody inherently also binds (cross-reactive) to the claimed human insulin-like growth factor T receptor IGF-IR.

With respect to the patent documents cited above (the '996, '821' '039, '841, and '064 patents), the Examiner is requiring that Applicants show that the prior art antibody is different from the claimed antibody.

The '996 patent is directed to anti-EGFR monoclonal antibodies for diagnosing and treating cancer; the '821 patent is directed to 1A7 anti-idiotype anti-GD2 antibody for diagnosing and treating cancer; the '039 patent is directed to Fv chain regions of antibody B1, B3 and B5 directed against Lewis' antigen and its use for targeting immunotoxins to tumors in diagnosing and treating cancer; the '841 patent is directed to NOK-NOK5 antibodies directed against the human Fas Ligand

for hepatitis treatment; and the '064 is directed to immunoglobulin variable domain sequences and their use for the preparation of nucleic acids or peptide libraries.

Applicants submit herein as Exhibit "C" a comparison of sequences of the murine "7C10" and humanized "A2CHM" IGF-IR antibodies of the present invention with the sequences disclosed in the references listed by the Examiner. The summary table, 14 and 15 of the Appendix, demonstrates that the maximum of identity found for the 6 complementary determining regions (CDR) of all the antibodies disclosed in the cited prior art is less than 65% for 1A7 antibody.

Furthermore, Applicants submit that it is well known by one of skill in the art that the specificity of an antibody is determined by the amino acid sequences of the CDR1, 2 and 3 of the heavy and the light chain. Thus, the complementary determining regions of the heavy and light chain variable regions will be considered as the principal molecular structural feature of a monoclonal antibody. In support of Applicant's position, attached hereto as Exhibit "D" "Interpreting Sameness of Monoclonal Antibody Products Under the Orphan Drug Regulations," (1999), *In* Guidance for Industry, Chapter IV, part B, first paragraph, relative to monoclonal antibody products.

In view of the foregoing comparison, and in light of the information provided in the accompanying exhibits, Applicants submit that the cited references fail to anticipate the claimed antibody. Nevertheless, Applicants have amended the claims to delete any reference to "percent identity" in order to expedite allowance of the claims. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 102(b) and 35 U.S.C. § 102(e).

Rejection under 35 U.S.C. § 103

Claims 1, 11-14 and 29 have been rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,935,821 in view of U.S. Patent No. 6,180,370B. Specifically, the Examiner purports that it would have been obvious to one having ordinary skill in the art at the time the invention was made to produce humanized or chimeric antibody that comprises the light and heavy chain constant region derived from man as taught by the '370 patent using the donor monoclonal antibody that comprises the light chain having a sequence such as SEQ ID NO:66 that is 93.4% identical to the claimed SEQ ID NO:54, as taught by the '821 patent. Applicants respectfully traverse the rejection.

As discussed *supra* the sequence comparison submitted herewith demonstrates that the maximum of identity found for the 6 complementary determining regions (CDR) of all the antibodies disclosed in the cited prior art is less than 65% for 1A7 antibody. Therefore, the claims cannot be *prima facie* obvious over U.S. Patent No. 5,935,821 in view of U.S. Patent No. 6,180,370B.

Claims 1, 24-28, 31-37, 43-52 and 54 are rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,891,996, as evidence by Rodeck et al, in view of U.S. Patent No. 6,342,219 B1 and U.S. Patent No. 6,235,883 or Ciardiello et al. Specifically, the Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the antibody that binds to human insulin-like growth factor I receptor as taught by the '996 patent with the antibody that binds to human epidermal growth factor receptor such as monoclonal C225 or humanized or human monoclonal antibody derived from m C225 alone or in combination with cytotoxic or cytostatic agent or

chemotherapeutic agent for treating cancer as purportedly taught by the '883 patent or Ciardiello et al. Further, the Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to pegylate any antibody fragment to increase the half-life of the antibody fragment as taught by the '219 patent. Applicants respectfully traverse the rejection.

Again, the primary patent cited in this rejection, the '996 patent, is directed to anti-EGFR monoclonal antibodies for diagnosing and treating cancer. The '966 patent does not teach or suggest an isolated antibody, or binding fragment thereof, comprising a light chain complementarity determining region (CDR) comprising SEQ ID Nos. 2, 4, and 6; and a heavy chain complementarity determining region (CDR) comprising SEQ ID Nos. 8, 10 and 12, wherein the antibody, or binding fragment thereof, binds to human insulin-like growth factor I receptor (IGF-IR) and inhibits binding of IGF1 and/or IGF2 to said IGF-IR. None of the references cited by the Examiner teach or suggest the combination of sequences as recited in amended claim 1. Accordingly, it would not have been obvious to produce the antibody or binding fragment thereof absent the information disclosed in the present specification. Applicant maintains that there is no suggestion to combine the references in the manner suggested by the Examiner to achieve the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 29-30, 36-39, 43 and 53 are rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,891,996, as evidence by Rodeck et al, in view of U.S. Patent No. 6,342,219 B1. Specifically, the Examiner purports that it would have been obvious to one of ordinary sill in the art at the time the invention

was made to substitute the anti-VEGF antibody in the immunoconjugate of the composition as purportedly taught by the '219 patent for the antibodies such as humanized, chimeric and monoclonal antibodies against human EGF receptor as taught by the '966 patent for a composition comprising antibody that binds to human insulin-like growth factor I receptor coupled chemically to vinblastine, vincristine, vindescine or derivative thereof or toxin. Further, the Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to put the mAb as taught by the '996 patent in a kit as taught by the '219 patent because a kit will allow for ease of use for the practitioner since purportedly all the necessary reagents, standard and instructions for use are included in a kit as taught by '219.

For the reasons set forth *supra*, Applicants submit that it would not have been obvious to produce the antibody or binding fragment thereof as claimed in the invention, absent the information disclosed in the present specification. Applicant maintains that there is no suggestion to combine the references in the manner suggested by the Examiner to achieve the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 1, 30 and 36 are rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,891,996, as evidence by Rodeck et al, in view of Traxler et al. Specifically, the Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the antibody that binds to human insulin-like growth factor I receptor as purportedly taught by the '996 patent with the various EGF receptor specific tyrosin kinase inhibitor such as dianilinophthalimide (CGP 52411) or CGP53353 or

phenylaminoquinazoline (PD153035) or phenylamino-pyrido-pyrimidines as purportedly taught by Traxler et al.

For the reasons set forth *supra*, Applicants submit that it would not have been obvious to produce the antibody or binding fragment thereof as claimed in the invention, absent the information disclosed in the present specification. Applicant maintains that there is no suggestion to combine the references in the manner suggested by the Examiner to achieve the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawn

Claims 1, 30-32 and 40 are rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,891,996, as evidence by Rodeck et al, in view of U.S. Patent No. 6,235,883 and Traxler et al. Specifically, The Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the anti-EGFR antibody or binding fragment thereof as taught by the '883 patent, the anti-human insulin-like growth factor I receptor or binding fragment thereof as taught by the '996 patent with the various tyrosine kinase receptor inhibitor such as dianilinophthalimide (CGP 52411) or CGP53353 or phenylaminoquinazoline (PD153035) or phenylamino-pyrido-pyrimidines as purportedly taught by Traxler et al.

For the reasons set forth *supra*, Applicants submit that it would not have been obvious to produce the antibody or binding fragment thereof as claimed in the invention, absent the information disclosed in the present specification. Applicant maintains that there is no suggestion to combine the references in the manner suggested by the Examiner to achieve the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawnClaims 1, 30, and 42

are rejected under 35 U.S.C. § 103(a) as purportedly unpatentable over U.S. Patent No. 5,891,996, as evidence by Rodeck et al, in view of U.S. Patent No. 6,949,245 or Baselga et al. Specifically, the Examiner purports that it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the antibody that binds specifically to human EGF receptor and cross-react with human insulin-like growth factor I receptor as taught by the '996 patent with the human Mab 4D5 recombinant human Mab HER2 or binding fragment thereof as purportedly taught by the '245 patent or the trastuzumab humanized monoclonal antibody or binding fragment thereof that binds to HER2 with high affinity as taught by Baselga et al for treatment of cancer.

For the reasons set forth *supra*, Applicants submit that it would not have been obvious to produce the antibody or binding fragment thereof as claimed in the invention, absent the information disclosed in the present specification. Applicant maintains that there is no suggestion to combine the references in the manner suggested by the Examiner to achieve the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawn.

It is believed that a 2-month extension of time and the accompanying fee is necessary to make this submission timely. If additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time

are hereby petitioned under 37 C.F.R. §1.136(a), and any fees required therefore (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: 7/2/06

By: _____
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1: Nature. 1998 Jul 23;394(6691):395-9. Related Articles, Links

nature

Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor.

Garrett TP, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW.

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The type-1 insulin-like growth-factor receptor (IGF-1R) and insulin receptor (IR) are closely related members of the tyrosine-kinase receptor superfamily. IR is essential for glucose homeostasis, whereas IGF-1R is involved in both normal growth and development and malignant transformation. Homologues of these receptors are found in animals as simple as cnidarians. The epidermal growth-factor receptor (EGFR) family is closely related to the IR family and has significant sequence identity to the extracellular portion we describe here. We now present the structure of the first three domains of IGF-IR (L1-Cys-rich-L2) determined to 2.6 A resolution. The L domains each consist of a single-stranded right-handed beta-helix. The Cys-rich region is composed of eight disulphide-bonded modules, seven of which form a rod-shaped domain with modules associated in an unusual manner. The three domains surround a central space of sufficient size to accommodate a ligand molecule. Although the fragment (residues 1-462) does not bind ligand, many of the determinants responsible for hormone binding and ligand specificity map to this central site. This structure therefore shows how the IR subfamily might interact with their ligands.

PMID: 9690478 [PubMed - indexed for MEDLINE]

J Clin Invest. 1995 Apr;95(4):1897-905. Related Articles, Links

Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin.

Wu X, **Fan Z**, **Masui H**, **Rosen N**, **Mendelsohn J**.

Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, USA.

Both EGF and insulin, or IGF, stimulate the growth of many cell types by activating receptors that contain tyrosine kinase activities. A monoclonal antibody (mAb 225) against the EGF receptor produced in this laboratory has been shown to competitively inhibit EGF binding and block activation of receptor tyrosine kinase. Here we report that a human colorectal carcinoma cell line, DiFi, which expresses high levels of EGF receptors on plasma membranes, can be induced to undergo G1 cell cycle arrest and programmed cell death (apoptosis) when cultured with mAb 225 at concentrations that saturate EGF receptors. Addition of IGF-1 or high concentrations of insulin can delay apoptosis induced by mAb 225, while the G1 arrest cannot be reversed by either IGF-1 or insulin. Insulin/IGF-1 cannot activate EGF receptor tyrosine kinase that has been inhibited by mAb 225. Moreover, an mAb against the IGF-1 receptor, which has little direct effect on DiFi cell growth, can block the capacity of insulin/IGF-1 to delay apoptosis induced by mAb 225, suggesting that the insulin/IGF-1-mediated delay of apoptosis is acting through the IGF-1 receptor. In contrast, insulin/IGF-1 cannot delay the apoptosis caused by the DNA damaging agent, cisplatin. The results indicate that EGF receptor activation is required both for cell cycle progression and for prevention of apoptosis in DiFi cells, and that a signal transduction pathway shared by receptors for insulin/IGF-1 and EGF may be involved in regulating apoptosis triggered by blockade of the EGF receptor.

PMID: 7706497 [PubMed - indexed for MEDLINE]

APPENDIX

Alignment of the variable sequences of the heavy and light chains of the antibodies disclosed in the patent documents US 5,608,039; US 5,891,996; US 5,935,821 and US 6,068,841 with the murine 7C10 and the humanized A2CHM antibodies of the present invention.

Comparison 7C10 versus MabB1 (US 5,608,039)

```
            Quality:    285            Length:    138
              Ratio:  2.436              Gaps:      3
 Percent Similarity: 56.034   Percent Identity: 49.138


                        .         .       H1.       .         .
FvH_MabB1     1 EVQLVESGGGLVKPGGSLKLSCAASGFIFSDNYMY.WVRQTPEKRLEWVA 49
                :||| ||| |||||  || |.|. .|:  .  |:: |:|| |  :|||.
VH_7C10       1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

                    H2  .         .         .         .         .
FvH_MabB1     50 TISDGGTYIDYSDSVKGRFTISRDNAKNNLYLQMSSLRSEDTGMYYCGRS 99
                  ||  ||  .|  |.| | .|.|| .||  :|.:.|. .|||  ||| |
VH_7C10       51 YISYDGTN.NYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCAR. 98

                  H3      .         .         .
FvH_MabB1    100 PIYYDYAPFTYWGQGTLVTVSAAKTTPPSVYPLAPGSA 137
                    |    | |||||| .|||.
VH_7C10       99 ...YGRVFFDYWGQGTTLTVSS..............  117


            Quality:    490            Length:    125
              Ratio:  4.336              Gaps:      0
 Percent Similarity: 85.841   Percent Identity: 82.301


                        .         .       . L1      .         .
VL_7C10        1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
                 ||.||| |||||||||||||||||||.:|||.| ||| |:||||||||
FvL_MabB1      1 DVVMTQTPLSLPVSLGDQASISCRSSQNLVHSDGKTYLHWFLQKPGQSPT 50

                    L2.         .         .         .       L3.
VL_7C10       51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
                 |||||||||  |||||||||||||| |||| |||||||||:| | .|||
FvL_MabB1     51 LLIYKVSNRFSGVPDRFSGSGSGTDFILKISRVEAEDLGVYFCSQSTHVP 100

                        .         .
VL_7C10      101 WTFGGGTKLEIKR.............  113
                  ||| |||||:||
FvL_MabB1    101 LTFGAGTKLELKRADAAPTVSIFPP 125
```

Comparison 7C10 versus MabB3 (US 5,608,039)

```
          Quality:    277            Length:    121
            Ratio:  2.368              Gaps:      4
 Percent Similarity: 55.652   Percent Identity: 47.826


                        .         .          .H1       .         .
VH_MabB3      1 DVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMY.WVRQTPEKRLEWVA 49
                ||.| ||| |||.|  || |.|. .|:. .  |:: |:|| |  :|||.
VH_7C10       1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

                   H2     .         .          .         .         .
VH_MabB3     50 YISNDDSSAAYSDTVKGRFTISRDNARNTLYLQMSRLKSEDTAIYSCARG 99
                ||| | ..  |  ..| | .|.|| .:|  :|.:. . .|||| | |||
VH_7C10      51 YISYDGTN.NYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCAR. 98

                  H3      .         .
VH_MabB3    100 LAWG.AWFAYWGQGTLVTVSS 119
                  :|  .| |||||| .||||
VH_7C10      99 ..YGRVFFDYWGQGTTLTVSS 117


          Quality:    541            Length:    113
            Ratio:  4.830              Gaps:      0
 Percent Similarity: 93.750   Percent Identity: 92.857


                        .         .          .L1       .         .
VL_7C10       1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
                |||||| |||||||||||||||||| |||||||||:||||||||||||
VL_MabB3      1 DVLMTQSPLSLPVSLGDQASISCRSSQIIVHSNGNTYLEWYLQKPGQSPK 50

                     L2  .         .          .         .       L3.
VL_7C10      51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
                ||||||||| ||||||||||||||||||| ||||||||||||||||||||
VL_MabB3     51 LLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVP 100

                        .
VL_7C10     101 WTFGGGTKLEIKR 113
                .||| |||||||
VL_MabB3    101 FTFGSGTKLEIK. 112
```

Comparison A2CHM versus humB3 (US 5,608,039)

```
          Quality:    305            Length:    121
            Ratio:  2.607              Gaps:      4
 Percent Similarity: 60.870   Percent Identity: 53.913

                        .         .          .H1       .         .
VH_A2CHM      1 QVQLQESGPGLVKPSETLSLTCTVSGYSITGGYLWNWIRQPPGKGLEWIG 50
                |.| ||| |.|.|  .| |.|  ||:. .  |:: |:|| |||||||:
VH_humB3      1 DVKLVESGGGVVQPGRSLKLSCATSGFTFSDYYMY.WVRQAPGKGLEWVA 49

                  H2    .         .          .         .         .
VH_A2CHM     51 YISYDGTN.NYKPSLKDRVTISRDTSKNQFSLKLSSVTAADTAVYYCAR. 98
                ||| | ..  |  ..| | ||||| |||   |.:. . | |||:| |||
VH_humB3     50 YISNDDSSAAYSDTVKGRFTISRDNSKNTLYLQMNRLRAEDTAIYSCARG 99

                  H3     .         .
VH_A2CHM     99 ..YGRVFFDYWGQGTLVTVSS 117
                  :|  .| ||||||||||||
VH_humB3    100 LAWG.AWFAYWGQGTLVTVSS 119


          Quality:    559            Length:    112
            Ratio:  4.991              Gaps:      0
 Percent Similarity: 95.536   Percent Identity: 93.750

                        .         .          .L1       .         .
VL_A2CHM      1 DIVMTQSPLSLPVTPGEPASISCRSSQSIVHSNGNTYLQWYLQKPGQSPQ 50
                |:.|||||||||||||||||||||| |||||||||:||||||||||
VL_humB3      1 DVLMTQSPLSLPVTPGEPASISCRSSQIIVHSNGNTYLEWYLQKPGQSPQ 50

                    L2    .         .          .         .       L3.
VL_A2CHM     51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP 100
                ||||||||| ||||||||||||||||||||||||||||||||||||||||
VL_humB3     51 LLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVP 100

                        .
VL_A2CHM    101 WTFGQGTKVEIK 112
                .||||||||||||
VL_humB3    101 FTFGQGTKVEIK 112
```

Comparison 7C10 versus MabB5 (US 5,608,039)

```
            Quality:    285            Length:    126
              Ratio:  2.436              Gaps:      3
 Percent Similarity: 55.172   Percent Identity: 48.276


                          .          .          .H1       .          .
VH_7C10       1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50
                :|.| ||| |||.|  || |.|. .|:. .  |:: |:|| |  :|||.
VH_MabB5      1 EVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMY.WVRQTPEKRLEWVA 49

                   .H2       .          .          .          .
VH_7C10      51 YISYDGTNNYKP.SLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCAR. 98
                |||  | . | | ..| | .| || .||  :|.:. . .|||| |||||
VH_MabB5     50 YISNGGGSTYYPDTVKGRFTIDRDNAKNTLYLQMSRLKSEDTAMYYCARG 99

                  .          .
VH_7C10      99 .YGRVFFDYWGQGTTLTVSS..... 117
                   .| |||||| .||||
VH_MabB5    100 LSDGSWFAYWGQGTLVTVSSGGGGSG 125


            Quality:    550            Length:    125
              Ratio:  4.867              Gaps:      0
 Percent Similarity: 94.690   Percent Identity: 92.920


                          .          .          .L1       .          .
VL_7C10       1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
                |||:|| ||||||||||||||||||||||||||||||:||||||||||
VL_MabB5      1 DVLLTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPK 50

                     L2    .          .          .          .      L3 .
VL_7C10      51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
                |||||||||  ||||||||||||||||||| ||||||||||||||||||
VL_MabB5     51 LLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVP 100

                          .          .
VL_7C10      101 WTFGGGTKLEIKR..... 113
                 .||| ||||||||
VL_MabB5     101 FTFGSGTKLEIKRADAAPTVSIFPP 125
```

Comparison 7C10 x anti-EGFR (US 5,891,996)

```
            Quality:    279            Length:    124
              Ratio:  2.385              Gaps:      3
 Percent Similarity: 57.759   Percent Identity: 48.276


                     .         .         .H1       .         .
VH_EGFR    1 QVQLQQPGAELVKPGASVKLSCKASGYTFTNYYIY.WVKQRPGQGLEWIG 49
             |||||: |  ||||  |. |.|  .||. |  |:: |::| ||  |||.|
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

                H2    .         .         .         .         .
VH_EGFR   50 GINPTSGGSNFNEKFKTKATLTVDESSTTAYMQLSSLTSEDSAVYYCTRQ 99
              |.   | .|:    | : .:| | |    ::.|.|.|.||.| ||| |
VH_7C10   51 YIS.YDGTNNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCARY 99

                H3    .         .
VH_EGFR  100 GLWFDSDGRGFDFWGQGTTLTVSS 123
             |  |      ||:||||||||||||
VH_7C10  100 GRVF......FDYWGQGTTLTVSS 117


           Quality:    550            Length:    114
             Ratio:  4.867              Gaps:      0
 Percent Similarity: 92.920   Percent Identity: 92.920


                     .         .         .L1       .         .
VL_7C10    1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
             |||||||||||||||||||||||||.|||||||||| ||||||||||
VL_EGFR    1 DVLMTQIPLSLPVSLGDQASISCRSSQNIVHSNGNTYLDWYLQKPGQSPN 50

                 L2  .         .         .         .       L3.
VL_7C10   51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
             |||||||||  |||||| |||||||||||||| |||||||||||||| ||||
VL_EGFR   51 LLIYKVSNRFSGVPDRFRGSGSGTDFTLKISRVEAEDLGVYYCFQYSHVP 100

                    .
VL_7C10  101 WTFGGGTKLEIKR. 113
             |||||||||||||
VL_EGFR   101 WTFGGGTKLEIKRA 114
```

Comparison 7C10 versus 1A7 (US 5,935,821)

```
            Quality:    357              Length:    153
              Ratio:  3.051                Gaps:      2
 Percent Similarity: 68.103   Percent Identity: 61.207


                .         .         .         .         .
VH_1A7     1 MAVLGLLFCLVTFPSCVLSQVQVKESGPFLVPPSQSLSITCTVSGFSLT. 49
                            ||..|||| || ||||||:||.|.|:|:|
VH_7C10    1 ..................DVQLQESGPGLVKPSQSLSLTCSVTGYSITG 31

             H1       .         . H2      .         .         .
VH_1A7    50 TYGVSWIRQPPGKGLEWLGAIWGDGTTNYHSALISRLSISKDNSKSQVFL 99
              |  .|||| ||  |||:| |  ||| ||  .|  |:||.:| ||.| ||
VH_7C10   32 GYLWNWIRQFPGNKLEWMGYISYDGTNNYKPSLKDRISITRDTSKNQFFL 81

                    .       H3.         .         .         .
VH_1A7   100 KLNSLQTDDTATYYCAKLGNYDALDYWGQGTSVTVSSAKTTPPPVYPLVP 149
             ||||.  :|||||||| |     |||||||..||||
VH_7C10   82 KLNSVTNEDTATYYCARYGRV.FFDYWGQGTTLTVSS.............. 117

            Quality:    552              Length:    149
              Ratio:  4.885                Gaps:      0
 Percent Similarity: 93.805   Percent Identity: 92.920


                .         .         .         .         .
VL_7C10    1 ..................DVLMTQIPLSLPVSLGDQASISCRSSQSIVH 31
                             || ||| ||||||||||||||||||||||||
VL_1A7     1 MKLPVRLLVLMFWIPASSDDVFMTQTPLSLPVSLGDQASISCRSSQSIVH 50

             L1       .         .     L2  .         .         .
VL_7C10   32 SNGNTYLQWYLQKPGQSPKLLIYKVSNRLYGVPDRFSGSGSGTDFTLKIS 81
             |||||||:||||||||| |||| ||||  ||||||||||||||||||||
VL_1A7    51 SNGNTYLEWYLQKPGQSPNLLIYFVSNRFSGVPDRFSGSGSGTDFTLKIS 100

                    .     L3  .         .         .
VL_7C10   82 SVEAEDLGVYYCFQGSHVPWTFGGGTKLEIKR.................. 113
              ||||||||||||||||||||||||||||||
VL_1A7   101 RVEAEDLGVYYCFQGSHVPWTFGGGTKLEIKRADAAPTVSIFPPSSKLG 149
```

Comparison 7C10 versus NOK1 (US 6,068,841)

```
          Quality:    286            Length:    122
            Ratio:  2.444              Gaps:      4
 Percent Similarity: 58.261   Percent Identity: 49.565


                        .          .        .H1       .          .
VH_NOK1    1 .VQLQESGPELVKPGASVKISCKASGYAFSSSWM.NWVKQRPGKGLEWIG 48
               |||||||| |||| |. :.|  .||. .  :: ||::| ||  |||.|
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

               H2     .          .          .          .          .
VH_NOK1   49 RIYPGDGDTNDNGKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARS 98
               |   ||  |     | : .:| | | .  ::.|.|.|.||.| |:|||
VH_7C10   51 YI.SYDGTNNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCAR. 98

               H3    .          .
VH_NOK1   99 YYYDGSPWFTYWGQGTTVTVSS 120
               | |  .| |||||||.||||
VH_7C10   99 ..Y.GRVFFDYWGQGTTLTVSS 117


          Quality:    467            Length:    113
            Ratio:  4.133              Gaps:      0
 Percent Similarity: 82.301   Percent Identity: 78.761


                        .          .        .L1       .          .
VL_7C10    1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
             |||||| ||||||.:|||||||||:|..|:..|.| ||| | ||||||||.
VL_NOK1    1 DVLMTQTPLSLPVNIGDQASISCKSTKSLLNSDGFTYLGWCLQKPGQSPQ 50

               L2    .          .          .          .      L3 .
VL_7C10   51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
             |||| ||||  ||||||||||||||||||| ||||||||||||| .:.|
VL_NOK1   51 LLIYLVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQSNYLP 100

                        .
VL_7C10  101 WTFGGGTKLEIKR 113
             ||| ||||||||
VL_NOK1  101 LTFGSGTKLEIKR 113
```

Comparison 7C10 versus NOK2 (US 6,068,841)

```
            Quality:    291              Length:    120
              Ratio:  2.487                Gaps:      3
 Percent Similarity: 58.261   Percent Identity: 48.696


                     .          .          .H1       .          .
VH_NOK2    1 .VQLQQSGAELVRPGTSVKMSCKAAGYTFT.NYWIGWVKQRPGHGLEWIG 48
              ||||:||  ||:|  |. :.|   ||. |  |   |::| ||. |||.|
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

                  H2     .          .          .          .          .
VH_NOK2   49 YLYPGGLYTNYNEKFKGKATLTADTSSSTAYMQLSSLTSEDSAIYYCARY 98
               |:   |   ||    | : .:| ||| .  ::.|.|.|.||.| ||||||
VH_7C10   51 YISYDGT.NNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCARY 99

                H3      .          .
VH_NOK2   99 RDYDYAMDYWGQGTTVTVSS 118
                   :  ||||||||.||||
VH_7C10  100 GRVFF..DYWGQGTTLTVSS 117


            Quality:    467              Length:    113
              Ratio:  4.133                Gaps:      0
 Percent Similarity: 82.301   Percent Identity: 78.761


                     .          .          .L1       .          .
VL_7C10    1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
             |||||| |||||||.:||||||||:|..|:..|.| ||| | ||||||||.
VL_NOK2    1 DVLMTQTPLSLPVNIGDQASISCKSTKSLLNSDGFTYLGWCLQKPGQSPQ 50

                  L2     .          .          .          .       L3.
VL_7C10   51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
             |||| ||||  ||||||||||||||||||| ||||||||||| .:.|
VL_NOK2   51 LLIYLVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQSNYLP 100

                     .
VL_7C10  101 WTFGGGTKLEIKR 113
              ||| ||||||||
VL_NOK2  101 LTFGSGTKLEIKR 113
```

Comparison 7C10 versus NOK3 (US 6,068,841)

```
            Quality:    296              Length:    118
              Ratio:  2.552                Gaps:      2
 Percent Similarity: 56.522   Percent Identity: 46.957

                     .         .          .H1       .         .
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50
             |.|||||| |||| |. :.|  .||. .  :: ||::| ||  |||.|
VH_NOK3    1 .VKLQESGPELVKPGASVKISCKASGYAFSSSWM.NWVKQRPGKGLEWIG 48

              H2      .         .          .         .         .
VH_7C10   51 YI.SYDGTNNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCARY 99
              |   .|  ||    | : .:| | | .  ::.|.|.|.||.| |:||
VH_NOK3   49 RIYPVNGDTNYNGKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYFCATD 98

               H3     .
VH_7C10  100 GRVFFDYWGQGTTLTVSS 117
             |  :|| ||||||.||||
VH_NOK3   99 GYWYFDVWGQGTTVTVSS 116
```

Comparison 7C10 versus NOK4 (US 6,068,841)

```
           Quality:    549              Length:    119
             Ratio:  4.692                Gaps:      1
 Percent Similarity: 89.655   Percent Identity: 89.655


                  .         .         .  H1     .         .
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50
             |||||||||||||||||||||||||||| || ||||||||||||||||
VH_NOK4    1 .VQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMG 49

               H2    .         .         .         .         .
VH_7C10   51 YISYDGTNNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCA..R 98
             ||||||.||| ||||.||||||||||||||||||| |||||||||
VH_NOK4   50 YISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAVYY 99

              H3      .
VH_7C10   99 YGRVFFDYWGQGTTLTVSS 117
             |    |||||||||.||||
VH_NOK4  100 YDGSSFDYWGQGTTVTVSS 118


           Quality:    353              Length:    113
             Ratio:  3.152                Gaps:      1
 Percent Similarity: 68.750   Percent Identity: 62.500


                  .         .        .L1       .         .
VL_7C10    1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
             |:.:|| | || |||  .|.||||.|:  | | | .:: || ||||| ||
VL_NOK4    1 DIVLTQSPASLAVSLRQRATISCRASEG.VDSYGISFMHWYQQKPGQPPK 49

               L2    .         .         .         .       L3.
VL_7C10   51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
             ||||: |    ||| ||||||| ||||| |  |||:|   ||| | .  |
VL_NOK4   50 LLIYRASYLKSGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNEDP 99

                  .
VL_7C10  101 WTFGGGTKLEIKR 113
             |||||||||||||
VL_NOK4  100 WTFGGGTKLEIKR 112
```

Comparison 7C10 versus NOK5 (US 6,068,841)

```
           Quality:    297              Length:    119
             Ratio:  2.538                Gaps:      3
 Percent Similarity: 56.522   Percent Identity: 49.565


                     .         .         .H1       .         .
VH_NOK5    1 .VQLQESGAEPAKPGASVKMSCKASGYTFT.TYWMHWVKQRPGQGLEWIG 48
             |||||||    ||  |. :.|  .||. |  |  .|::| ||  |||.|
VH_7C10    1 DVQLQESGPGLVKPSQSLSLTCSVTGYSITGGYLWNWIRQFPGNKLEWMG 50

               H2     .         .         .         .         .
VH_NOK5   49 YINPSSGYTEYNQKFKDKATLTADKSSSTAYMQLISLTSEDSAVYYCARR 98
             ||.   |   |    ||: .:| | | .  ::.| |.|.||.| |||||
VH_7C10   51 YIS.YDGTNNYKPSLKDRISITRDTSKNQFFLKLNSVTNEDTATYYCARY 99

              H3     .
VH_NOK5   99 GNYYYFDYWGQGTTVTVSS 117
             |   :||||||||||.||||
VH_7C10  100 GR.VFFDYWGQGTTLTVSS 117


           Quality:    352              Length:    113
             Ratio:  3.352                Gaps:      2
 Percent Similarity: 72.381   Percent Identity: 67.619


                     .         .        . L1      .         .
VL_7C10    1 DVLMTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLQWYLQKPGQSPK 50
             |||||| |  |||| ||. ...|:.|||:    ||  . || ||||||||
VL_NOK5    1 DVLMTQTPKFLPVSAGDRVTMTCKASQSV....GNN.VAWYQQKPGQSPK 45

                 L2    .         .         .         .       L3.
VL_7C10   51 LLIYKVSNRLYGVPDRFSGSGSGTDFTLKISSVEAEDLGVYYCFQGSHVP 100
             ||||  |||  ||||||.|||||||||  ||||: ||| ||:| |    |
VL_NOK5   46 LLIYYTSNRYTGVPDRFTGSGSGTDFTFTISSVQVEDLAVYFCQQHYSSP 95

                    .
VL_7C10  101 WTFGGGTKLEIKR 113
             :||| |||||
VL_NOK5   96 YTFGSGTKLE... 105
```

Comparison Humanized VH_7C10 (SEQ ID NO: 83) versus SEQ ID NO: 39 (US 6,300,064)

```
Query 1 = SEQ ID NO: 39(US 6,300,064)
Sbjct   = SEQ ID NO: 83(Humanized VH_7C10)

Identities = 99/120 (82%), Positives = 101/120 (84%), Gaps = 5/120 (4%)

Query  1    QVQLQESGPGLVKPSETLSLTCTVXXXXXXXXXXXXX-RQPPGKGLEWIGYIYYSGSTNY
59
            QVQLQESGPGLVKPSETLSLTCTVSG SIS  Y     RQPPGKGLEWIGYI Y G+ NY
Sbjct  1    QVQLQESGPGLVKPSETLSLTCTVSGYSISGGYLWNWIRQPPGKGLEWIGYISYDGTNNY
60

Query  60   -PSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARWGGDGFYAMDYWGQGTLVTVSS
118
             PSLK RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR+G   F   DYWGQGTLVTVSS
Sbjct  61   KPSLKDRVTISVDTSKNQFSLKLSSVTAADTAVYYCARYGRVFF---DYWGQGTLVTVSS
117

In bold, CDR 1, 2 and 3 sequences
```

Multiple Sequence Alignment Results (Heavy Chains).

```
            1                                                  50
     ch_7c10 ~~~~~~~~~~ ~~~~~~~~~D VQLQESGPGL VKPSQSLSLT CSVTGYSITG
      VH_NOK4 ~~~~~~~~~~ ~~~~~~~~~~ VQLQESGPGL VKPSQSLSLT CSVTGYSITS
    a2chm_ch ~~~~~~~~~~ ~~~~~~~~~Q VQLQESGPGL VKPSETLSLT CTVSGYSITG
      VH_1A7 MAVLGLLFCL VTFPSCVLSQ VQVKESGPFL VPPSQSLSIT CTVSGFSLTT
      VH_NOK1 ~~~~~~~~~~ ~~~~~~~~~~ VQLQESGPEL VKPGASVKIS CKASGYAFSS
      VH_NOK3 ~~~~~~~~~~ ~~~~~~~~~~ VKLQESGPEL VKPGASVKIS CKASGYAFSS
      VH_NOK2 ~~~~~~~~~~ ~~~~~~~~~~ VQLQQSGAEL VRPGTSVKMS CKAAGYTFTN
      VH_NOK5 ~~~~~~~~~~ ~~~~~~~~~~ VQLQESGAEP AKPGASVKMS CKASGYTFTT
 VH_antiEGFR ~~~~~~~~~~ ~~~~~~~~~Q VQLQQPGAEL VKPGASVKLS CKASGYTFTN
    VH_MabB3 ~~~~~~~~~~ ~~~~~~~~~D VKLVESGGGL VQPGGSLKLS CATSGFTFSD
    VH_humB3 ~~~~~~~~~~ ~~~~~~~~~D VKLVESGGGV VQPGRSLKLS CATSGFTFSD
   FvH_MabB5 ~~~~~~~~~~ ~~~~~~~~~E VKLVESGGGL VQPGGSLKLS CATSGFTFSD
   FvH_MabB1 ~~~~~~~~~~ ~~~~~~~~~E VQLVESGGGL VKPGGSLKLS CAASGFIFSD

            51                                                 100
     ch_7c10 GYLWNWIRQF PGNKLEWMGY IS.YDGTNNY KPSLKDRISI TRDTSKNQFF
      VH_NOK4 GYYWNWIRQF PGNKLEWMGY IS.YDGSNNY NPSLKNRISI TRDTSKNQFF
    a2chm_ch GYLWNWIRQP PGKGLEWIGY IS.YDGTNNY KPSLKDRVTI SRDTSKNQFS
      VH_1A7 .YGVSWIRQP PGKGLEWLGA IW.GDGTTNY HSALISRLSI SKDNSKSQVF
      VH_NOK1 SWM.NWVKQR PGKGLEWIGR IYPGDGDTND NGKFKGKATL TADKSSSTAY
      VH_NOK3 SWM.NWVKQR PGKGLEWIGR IYPVNGDTNY NGKFKGKATL TADKSSSTAY
      VH_NOK2 YWI.GWVKQR PGHGLEWIGY LYPGGLYTNY NEKFKGKATL TADTSSSTAY
      VH_NOK5 YWM.HWVKQR PGQGLEWIGY INPSSGYTEY NQKFKDKATL TADKSSSTAY
 VH_antiEGFR YYI.YWVKQR PGQGLEWIGG INPTSGGSNF NEKFKTKATL TVDESSTTAY
    VH_MabB3 YYM.YWVRQT PEKRLEWVAY ISNDDSSAAY SDTVKGRFTI SRDNARNTLY
    VH_humB3 YYM.YWVRQA PGKGLEWVAY ISNDDSSAAY SDTVKGRFTI SRDNSKNTLY
   FvH_MabB5 YYM.YWVRQT PEKRLEWVAY ISNGGGSTYY PDTVKGRFTI DRDNAKNTLY
   FvH_MabB1 NYM.YWVRQT PEKRLEWVAT ISDGGTYIDY SDSVKGRFTI SRDNAKNNLY

            101                                                150
     ch_7c10 LKLNSVTNED TATYYCA... RYGRV...FF DYWGQGTTLT VSS~~~~~~~
      VH_NOK4 LKLNSVTTED TATYYCA.VY YYDGS...SF DYWGQGTTVT VSS~~~~~~~
    a2chm_ch LKLSSVTAAD TAVYYCA... RYGRV...FF DYWGQGTLVT VSS~~~~~~~
      VH_1A7 LKLNSLQTDD TATYYCAKLG NYDA.....L DYWGQGTSVT VSSAKTTPPP
      VH_NOK1 MQLSSLTSED SAVYFCARSY YY..DGSPWF TYWGQGTTVT VSS~~~~~~~
      VH_NOK3 MQLSSLTSED SAVYFCAT.. ....DGYWYF DVWGQGTTVT VSS~~~~~~~
      VH_NOK2 MQLSSLTSED SAIYYCARYR DY..D..YAM DYWGQGTTVT VSS~~~~~~~
      VH_NOK5 MQLISLTSED SAVYYCARRG NY.....YYF DYWGQGTTVT VSS~~~~~~~
 VH_antiEGFR MQLSSLTSED SAVYYCTRQG LWFDSDGRGF DFWGQGTTLT VSS~~~~~~~
    VH_MabB3 LQMSRLKSED TAIYSCARGL AWGA..W..F AYWGQGTLVT VSS~~~~~~~
    VH_humB3 LQMNRLRAED TAIYSCARGL AWGA..W..F AYWGQGTLVT VSS~~~~~~~
   FvH_MabB5 LQMSRLKSED TAMYYCARGL SDGS..W..F AYWGQGTLVT VSSGGGGSG~
   FvH_MabB1 LQMSSLRSED TGMYYCGRSP IYYD..YAPF TYWGQGTLVT VSAAKTTPPS
```

Multiple Sequence Alignment Results (Light Chains)

```
            1                                                       50
   a2chm_cl ~~~~~~~~~~ ~~~~~~~~~D IVMTQSPLSL PVTPGEPASI SCRSSQSIVH
   VL_humB3 ~~~~~~~~~~ ~~~~~~~~~D VLMTQSPLSL PVTPGEPASI SCRSSQIIVH
   VL_MabB3 ~~~~~~~~~~ ~~~~~~~~~D VLMTQSPLSL PVSLGDQASI SCRSSQIIVH
  FvL_MabB5 ~~~~~~~~~~ ~~~~~~~~~D VLLTQTPLSL PVSLGDQASI SCRSSQSIVH
VL_antiEGFR ~~~~~~~~~~ ~~~~~~~~~D VLMTQIPLSL PVSLGDQASI SCRSSQNIVH
     VL_1A7 MKLPVRLLVL MFWIPASSDD VFMTQTPLSL PVSLGDQASI SCRSSQSIVH
    cl_7c10 ~~~~~~~~~~ ~~~~~~~~~D VLMTQIPLSL PVSLGDQASI SCRSSQSIVH
  FvL_MabB1 ~~~~~~~~~~ ~~~~~~~~~D VVMTQTPLSL PVSLGDQASI SCRSSQNLVH
    VL_NOK2 ~~~~~~~~~~ ~~~~~~~~~D VLMTQTPLSL PVNIGDQASI SCKSTKSLLN
    VL_NOK1 ~~~~~~~~~~ ~~~~~~~~~D IQMTQSPSSL SASLGDRVTI SCRASQDI..
    VL_NOK5 ~~~~~~~~~~ ~~~~~~~~~D VLMTQTPKFL PVSAGDRVTM TCKASQSV..
    VL_NOK4 ~~~~~~~~~~ ~~~~~~~~~D IVLTQSPASL AVSLRQRATI SCRASEG.VD

            51                                                      100
   a2chm_cl SNGNTYLQWY LQKPGQSPQL LIYKVSNRLY GVPDRFSGSG SGTDFTLKIS
   VL_humB3 SNGNTYLEWY LQKPGQSPQL LIYKVSNRFS GVPDRFSGSG SGTDFTLKIS
   VL_MabB3 SNGNTYLEWY LQKPGQSPKL LIYKVSNRFS GVPDRFSGSG SGTDFTLKIS
  FvL_MabB5 SNGNTYLEWY LQKPGQSPKL LIYKVSNRFS GVPDRFSGSG SGTDFTLKIS
VL_antiEGFR SNGNTYLDWY LQKPGQSPNL LIYKVSNRFS GVPDRFRGSG SGTDFTLKIS
     VL_1A7 SNGNTYLEWY LQKPGQSPNL LIYFVSNRFS GVPDRFSGSG SGTDFTLKIS
    cl_7c10 SNGNTYLQWY LQKPGQSPKL LIYKVSNRLY GVPDRFSGSG SGTDFTLKIS
  FvL_MabB1 SDGKTYLHWF LQKPGQSPTL LIYKVSNRFS GVPDRFSGSG SGTDFILKIS
    VL_NOK2 SDGFTYLGWC LQKPGQSPQL LIYLVSNRFS GVPDRFSGSG SGTDFTLKIS
    VL_NOK1 ...SNYLNWY QQKPDGTVKL LIYYTSRLHS GVPSRFSGSG SGTDYSLTIS
    VL_NOK5 ...GNNVAWY QQKPGQSPKL LIYYTSNRYT GVPDRFTGSG SGTDFTFTIS
    VL_NOK4 SYGISFMHWY QQKPGQPPKL LIYRASYLKS GVPARFSGSG SRTDFTLTID

            101                                                     149
   a2chm_cl RVEAEDVGVY YCFQGSHVPW TFGQGTKVEI K~~~~~~~~~ ~~~~~~~~~
   VL_humB3 RVEAEDVGVY YCFQGSHVPF TFGQGTKVEI K~~~~~~~~~ ~~~~~~~~~
   VL_MabB3 RVEAEDLGVY YCFQGSHVPF TFGSGTKLEI K~~~~~~~~~ ~~~~~~~~~
  FvL_MabB5 RVEAEDLGVY YCFQGSHVPF TFGSGTKLEI KRADAAPTVS IFPP~~~~~
VL_antiEGFR RVEAEDLGVY YCFQYSHVPW TFGGGTKLEI KRA~~~~~~~ ~~~~~~~~~
     VL_1A7 RVEAEDLGVY YCFQGSHVPW TFGGGTKLEI KRADAAPTVS IFPPSSKLG
    cl_7c10 SVEAEDLGVY YCFQGSHVPW TFGGGTKLEI KR~~~~~~~~ ~~~~~~~~~
  FvL_MabB1 RVEAEDLGVY FCSQSTHVPL TFGAGTKLEL KRADAAPTVS IFPP~~~~~
    VL_NOK2 RVEAEDLGVY YCFQSNYLPL TFGSGTKLEI KR~~~~~~~~ ~~~~~~~~~
    VL_NOK1 NLEPEDIATY FCQQYSEFPW TFGGGTKLEI KR~~~~~~~~ ~~~~~~~~~
    VL_NOK5 SVQVEDLAVY FCQQHYSSPY TFGSGTKLE~ ~~~~~~~~~~ ~~~~~~~~~
    VL_NOK4 PVEADDAATY YCQQNNEDPW TFGGGTKLEI KR~~~~~~~~ ~~~~~~~~~
```

Table summarizing the identity and similarity percentages obtained for heavy and/or light chain, for each CDR and the 6 CDR

Comparison with 7C10

		VH %	VL %	H+L %	H1	H2	H3	L1	L2	L3	CDR %
MabB1 5,608,039	id	49.1	82.3	70.7	1/10	4/10	4/12	10/16	5/7	4/8	43.0
	sim	56.0	85.8	65.5	4/10	4/10	4/12	12/16	5/7	4/8	50.8
	gaps	3	0		1	1	2	0	0	0	
MabB3 5,608,039	id	47.8	92.9	70.0	1/10	4/10	5/13	14/16	5/7	7/8	56.3
	sim	55.7	93.8	74.4	4/10	4/10	6/132	15/16	5/7	7/8	64.1
	gaps	4	0		1	1	4	0	0	0	
MabB5 5,608,039	id	48.3	92.9	70.3	1/10	4/10	4/12	15/16	5/7	7/8	57.1
	sim	55.2	94.7	74.7	4/10	4/10	4/12	16/16	5/7	7/8	63.5
	gaps	3	0		1	0	2	0	0	0	
a-EGFR 5,891,996	id	48.3	92.9	70.3	3/10	3/10	5/16	14/16	5/7	7/8	55.2
	sim	57.8	92.9	75.1	5/10	3/10	6/16	14/16	5/7	7/8	59.7
	gaps	3	0		1	1	6	0	0	0	
1A7 5,935,921	id	61.2	92.9	76.9	3/10	5/9	4/11	15/16	4/7	8/8	63.9
	sim	68.1	93.8	80.8	5/10	5/9	5/11	16/16	4/7	8/8	70.5
	gaps	2	0		1	0	1	0	0	0	
NOK1 6,068,841	id	49.6	78.8	64.0	2/10	4/10	6/14	8/16	4/7	4/8	43.1
	sim	58.3	82.3	70.2	4/10	4/10	6/14	9/16	4/7	5/8	49.2
	gaps	4	0		1	1	4	0	0	0	
NOK2 6,068,841	id	48.7	78.8	63.6	3/10	3/10	5/12	8/16	4/7	3/8	41.3
	sim	58.3	82.3	70.2	3/10	4/10	6/12	9/16	4/7	4/8	47.6
	gaps	3	0		1	1	2	0	0	0	
NOK3 6,068,841	id	47.0	-	-	2/10	3/10	4/10	-	-	-	-
	sim	56.5	-	-	4/10	3/10	5/10	-	-	-	-
	gaps	2	-	-	1	1	0				
NOK4 6,068,841	id	89.7	62.5	76.3	8/10	8/9	5/12	5/16	1/7	3/8	48.4
	sim	89.7	68.8	79.4	8/10	8/9	5/12	8/16	2/7	3/8	54.8
	gaps	1	1		0	0	2	1	0	0	
NOK5 6,068,841	id	49.6	67.6	58.2	3/10	3/10	6/11	6/16	3/7	2/8	37.1
	sim	56.5	72.4	64.1	3/10	3/10	7/11	7/16	3/7	3/8	41.9
	gaps	3	2		1	1	1	5	0	0	

Comparison with A2CHM

		VH %	VL %	H+L %	H1	H2	H3	L1	L2	L3	CDR %
humB3 5,608,039	id	53.9	93.8	73.6	1/10	4/10	5/13	14/16	5/7	7/8	56.3
	sim	60.9	95.5	78.0	4/10	4/10	6/13	15/16	5/7	7/8	64.1
	gaps	4	0		1	1	2	0	0	0	
SEQ ID39 6,300,064	id	82			4/10	5/10	6/13				

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GUIDANCE FOR INDUSTRY[1]

Interpreting Sameness of Monoclonal Antibody Products Under the Orphan Drug Regulations

I. INTRODUCTION

The regulations implementing the Orphan Drug Act are codified in 21 CFR Part 316. FDA published the Proposed Rule for these regulations on January 29, 1991 (56 FR 3338) (Ref. 1) and the Final Rule on December 29, 1992 (57 FR 62076) (Ref. 2). One of the incentives for orphan drug development is the exclusive approval of a product for a period of seven years. During this seven year period, no approval will be given to a subsequent sponsor's marketing application for the same drug product for the same indication unless the subsequent product is shown by the sponsor to be clinically superior, as defined in 21 CFR 316.3 (b)(3). In determining whether or not two products would be considered the same, FDA recognized that different criteria were necessary for macromolecules versus small molecules [21 CFR 316.3(b)(13)]. Macromolecules include a variety of structures including proteins, nucleic acids, carbohydrates and closely related, complex, partly definable drugs such as vaccines or surfactants. The current definition of sameness for protein drugs [21 CFR 316.3(b)(13)(ii)(A)] however, does not adequately consider the unique nature of antibodies. The purpose of the present document is to describe FDA's current thinking on the criteria by which two monoclonal antibody products would be considered the same under the Orphan Drug Act and its implementing regulations.

II. BACKGROUND

21 CFR Part 316.3(b)(13)(ii) defines sameness for a macromolecule as "...a drug that contains the same principal molecular structural features (but not necessarily all of the same structural features) and is intended for the same use as a previously approved drug..." Two protein drugs would be considered the same "...if the only differences in structure between them were due to post-translational events or infidelity of translation or transcription or were minor differences in amino acid sequence ..." [21 CFR Part 316.3(b)(13)(ii)(A)]. For monoclonal antibody products, these definitions lay the groundwork for the determination of sameness but, because of the unique series of processes involved in creating an antibody molecule, additional guidance as to what would be considered the same under the Orphan Drug regulations is needed.

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[1]This guidance document represents the Agency's current thinking on the interpretation of the Orphan Drug regulations as they pertain to monoclonal antibodies. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of applicable statutes, regulations, or both.

An antibody molecule is composed of four polypeptide chains, two identical heavy (H) chains and two identical light (L) chains. Both heavy and light chains are divided into variable (V) and constant (C) regions. The V_H-V_L pairs confer specificity for antigen while the constant region of the heavy chain is responsible for effector functions such as, but not limited to, complement fixation and antibody dependent cellular cytotoxicity. The variable and constant regions were so named because amino acid sequence data showed that the amino terminal regions of heavy and light chains from different antibodies had different sequences while the carboxy terminal region amino acid sequences were the same within a given isotype (class or subclass). Subsequent analysis of variable region amino acid sequences defined three hypervariable regions (also known as complementarity determining regions or CDRs) each in the V_H and V_L regions which form the antigen binding site of the molecule (Ref. 3).

Antibody diversity is created by the use of multiple germline genes encoding variable regions and a variety of somatic events. The somatic events include recombination of variable gene segments with diversity (D) and joining (J) gene segments to make a complete V_H region and the recombination of variable and joining gene segments to make a complete V_L region. The recombination process itself is imprecise, resulting in the loss or addition of amino acids at the V(D)J junctions. These mechanisms of diversity occur in the developing B cell prior to antigen exposure. After antigenic stimulation, the expressed antibody genes in B cells undergo somatic mutation. Based on the estimated number of germline gene segments, the random recombination of these segments, and random V_H-V_L pairing, up to 1.6×10^7 different antibodies could be produced (Ref. 4). When other processes which contribute to antibody diversity (such as somatic mutation) are taken into account, it is thought that upwards of 1×10^{10} different antibodies could be generated (Ref. 5). Because of the many processes involved in generating antibody diversity, it is unlikely that independently derived monoclonal antibodies with the same antigen specificity will have identical amino acid sequences.

III. SCOPE

For the purpose of this document, a monoclonal antibody is a clonal product defined as any intact antibody, antibody fragment, conjugate, fusion protein, or bispecific antibody that contains a V_H - V_L pair where the CDRs form the antigen binding site. Antibody fragments or fusion proteins containing only constant region domains are not within the purview of this document.

The mechanisms generating antibody diversity are the same for all antibodies whether they are immortalized as monoclonal antibodies or purified from serum as polyclonal antibodies. The policy described in this document, however, will apply only to monoclonal antibody products.

Diversity of the T cell receptor is also generated by multiple T cell receptor specific germline genes and somatic events similar to those described for antibodies. The T cell receptor is membrane bound in its native functional form. The FDA anticipates the development of soluble T cell receptor products for therapeutic use. The interpretation of sameness for monoclonal antibody products in the present document will apply to soluble T cell receptor products.

IV. INTERPRETATION OF SAMENESS FOR MONOCLONAL ANTIBODY PRODUCTS

A. Structural Features of Antibodies

As described in section II above, antibodies have two functional regions, the variable region, which is responsible for antigen-specific binding, and the constant region which carries out effector functions. The variable region is divided into complementarity determining regions (CDR1, CDR2 and CDR3) and framework regions (FR1, FR2, and FR3). CDRs 1, 2, and 3 are delineated by amino acid positions 31-35, 50-65, and 95-102 for heavy chains and amino acid positions 24-34, 50-56, and 89-97 for light chains. While these amino acid positions define the boundaries of each CDR, the lengths of the CDRs can vary (Ref. 6). The CDRs create the antigen binding pocket of the molecule through the interaction between heavy and light chain variable regions while the framework regions provide the scaffolding on which the antigen binding pocket sits. The constant region is responsible for antibody effector functions but, has little influence on antibody specificity or affinity.

B. Sameness for Naked Monoclonal Antibody Products

The definition of sameness for a macromolecule is based on its principal molecular structure. For the purpose of determining sameness of naked monoclonal antibodies under the Orphan Drug Act and its implementing regulations, the complementarity determining regions of the heavy and light chain variable regions will be viewed by the FDA as the principal molecular structural feature of a monoclonal antibody product. The residues comprising the CDRs will be those stated in Section A. above as defined by Kabat et al. (Ref. 6).

The proposed interpretation of sameness for two monoclonal antibodies is that two monoclonal antibody drugs would be considered the same if the amino acid sequences of the complementarity determining regions were the same or if there were only minor amino acid differences between them. Other potentially important amino acid differences outside the complementarity determining regions, or differences due to glycosylation patterns or post translational modifications would not per se cause the products to be considered different unless the subsequent drug was shown to be clinically superior.

In the Orphan Drug Regulations Final Rule (57 FR 62076), Section II.B. (Summary of and Response to Comments; Sameness Versus Difference), comment 31 refers to a suggestion that a guidance document be developed to describe the differences in amino acid sequence of a protein which would be considered "minor". Now, as then, the FDA declines to provide examples for hypothetical situations. This determination would be made on a case-by-case basis. The types of information that would be useful in making such a determination include (but are not limited to) the sequence of the heavy and light chain variable regions of the product, any modifications made during the development process, and whether any particular residues have been established to be important for antigen binding.

C. Sameness for Antibody Conjugates, Fusion Proteins, and Bispecific Antibodies

Monoclonal antibody products can be conjugated by chemical methods with radionuclides, drugs, macromolecules, or other agents or can be made as fusion proteins. A monoclonal antibody fusion protein contains a V_H-V_L pair where one of these chains (usually V_H) and another protein are synthesized as a single polypeptide chain. These types of products differ from naked monoclonal antibodies in that they generally have an important additional functional element; the active moiety of a small molecule or the principal molecular structural feature of the conjugated or fused macromolecule.

The determination of sameness of monoclonal antibodies which have had relevant functional elements added will be based on a determination of sameness for the monoclonal antibody element and on a determination of sameness for the added relevant functional element (see, for example, 21 CFR 316.3(b)(13)(i) regarding small molecules and 21 CFR 316.3(b)(13)(ii) regarding macromolecules. A difference in any one of these elements may result in a determination that the molecules are different. Conversely, two monoclonal antibody conjugates or fusion proteins would be determined to be the same if both the CDR sequences of the antibody and the functional element of the conjugated molecule were the same.

Bispecific antibodies are generated by combining a heavy-light chain pair from a monoclonal antibody of one specificity with a heavy-light chain pair from a monoclonal antibody of a different specificity and therefore, have two different sets of CDRs. Two bispecific antibodies will be considered the same if both sets of CDRs are the same.

V. CHANGES IN ANTIBODY STRUCTURE THAT DO NOT CONSTITUTE DIFFERENCES BETWEEN TWO MONOCLONAL ANTIBODY PRODUCTS WITH THE SAME COMPLEMENTARITY DETERMINING REGIONS

Listed below are potential changes in areas outside the CDRs in monoclonal antibody products. For the purpose determining sameness of monoclonal antibodies under the Orphan Drug Act and its implementing regulations, such changes do not constitute differences between two monoclonal antibody products with the same CDRs unless the subsequent product is shown to be clinically superior.

A. Framework Regions

Framework region changes include, but are not limited to, humanizing a non-human derived monoclonal antibody or engineering certain framework residues that are important for antigen contact or for stabilizing the binding site.

B. Constant Region

Constant region differences include, but are not limited to, changing the class or subclass of the constant region, changing specific amino acid residues which might alter an effector function such as Fc receptor binding, or changing the species from which the constant region is derived.

C. Antibody Fragments

Intact monoclonal antibodies and antibody fragments with the same CDR sequences will not be considered different. This is consistent with FDA's policy regarding peptides and whole proteins as explained in Orphan Drug Regulations Final Rule (57 FR 62076), Section II. Summary of and Response to Comments, B. Sameness Versus Difference, comment 21 where it is stated that "...in order for a peptide that resembles a portion of a protein product to be considered a different drug, FDA will require a clear demonstration that the peptide is clinically superior to the entire protein."

VI. REFERENCES

1. Federal Register, 56 FR 3338 1/29/91, Orphan Drug Act: Proposed Rule, [Docket No. 85N-0483].
2. Federal Register, 57 FR 62076 12/29/92, Orphan Drug Act: Final Rule.
3. Kabat, E. A., "The Structural Basis of Antibody Complementarity". Advances in Protein Chemistry. Vol.32; pp. 1-75. 1978.
4. Max, E. E. Immunoglobulins: Molecular Genetics. In Fundamental Immunology. Third Edition (Ed. W. E. Paul) pp. 315-382, Raven Press, New York, New York, USA. 1993.
5. Burrows, P.D., Schroeder, H.W., and M. D. Cooper. B-Cell Differentiation in Humans. In Immunoglobulin Genes. Second Edition. (Ed. Honjo, T. and F. W. Alt) pp. 3-32, Academic Press, San Diego, California, USA. 1995.
6. Sequences of Proteins of Immunological Interest. Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman and C. Foeller (Eds.) U. S. Department of Health and Human Services, National Institute of Health, Bethesda, MD. 1991.

Guidance for Industry

Interpreting Sameness of Monoclonal Antibody Products Under the Orphan Drug Regulations

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication of the *Federal Register* notice announcing the availability of the draft guidance. Submit comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5600 Fishers Lane, rm 1061, Rockville, Md. 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from: Office of Communication, Training and Manufacturers Assistance (HFM-40), Center for Biologics Evaluation and Research, 1401 Rockville Pike, Rockville, MD 20852-1448 or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm

or

Office of Training and Communications, Division of Communications Management, Drug Information Branch (HFD-210), Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville MD 20852 or by calling 301-827-4573, or from the Internet at http://www.fda.gov/cder/guidance/index.htm

For questions on the content of the draft document contact Marjorie Shapiro, (301) 827-0850.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
Center for Drugs Evaluation and Research (CDER)
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